

## Reproductive biology of squaretail coralgrouper *Plectropomus areolatus* using age-based techniques

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(Received 5 September 2012, Accepted 28 January 2013)

The squaretail coral grouper *Plectropomus areolatus* was identified as a fast-growing, early maturing and relatively short-lived aggregation-spawning epinephelid. Examinations of sectioned otoliths found females and males first maturing at 2 and 3 years, respectively, suggesting protogynous hermaphroditism; however, no transitionals were observed in samples. Age distribution for the two sexes was similar and both were represented in the oldest age class; however, significant sex-specific differences in size-at-age were identified. Both sexes fully recruit into the fishery at age 4 years and reach 90% of asymptotic length by age 3 years. Underwater visual assessments, combined with the gonado-somatic indices, revealed a 5 month reproductive season, with interannual variability observed in the month of highest density within the spawning aggregation. Catch restrictions on adults during spawning times and at reproductive sites, combined with gear-based management and enhanced enforcement, are recommended to maintain spawning stocks. Based on the available evidence, the sexual pattern for this species is unresolved.

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Key words: age; Epinephelidae; growth; management; Pohnpei, Micronesia; sexual pattern.

## INTRODUCTION

The squaretail coral grouper *Plectropomus areolatus* (Rüppell, 1830) (Epinephelidae) is a widely distributed and commercially important medium-bodied grouper that occurs in lagoonal and seaward coral-rich areas of the tropical Indo-Pacific Ocean (Heemstra & Randall, 1993; Mapstone *et al.*, 2004; Williams *et al.*, 2008a; Hutchinson & Rhodes, 2010). As part of its reproductive life history, *P. areolatus* forms spawning aggregations of varying size (100 s–1000 s of individuals) throughout its distributional range that are frequent targets of large- and small-scale commercial

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and subsistence fisheries, including the regional south-east Asia-based live reef-food fish trade (Sluka, 2000; Sadovy *et al.*, 2003; Sadovy & Domeier, 2005; Rhodes & Tupper, 2008; Rhodes & Sadovy de Mitcheson, 2012). For *P. areolatus*, aggregation fishing can be severe (Hamilton *et al.*, 2011; Mangubhai *et al.*, 2011) and can quickly remove a substantial portion of the total aggregation (Wilson *et al.*, 2011), including from artisanal fishing (Hamilton *et al.*, 2012). As a consequence of dramatic, fisheries-induced reductions in population size throughout its range, the IUCN Red List designated *P. areolatus* as vulnerable (VU A4d) in 2008 (Thierry *et al.*, 2008; Sadovy de Mitcheson, *et al.*, 2012) suggesting a need for immediate and improved regional management. Although more has been learned about reproductive ecology of this species in recent years (Rhodes & Tupper, 2008; Hutchinson & Rhodes, 2010; Hamilton *et al.*, 2012), understanding of its reproductive development, spawning and age-based life history is still incomplete.

Among epinephelids, a wide range of sexual patterns has been identified (Sadovy de Mitcheson & Liu, 2008). Studies of sexual pattern in plectropomids have all reported protogyny (mature female to mature male sex change) (Ferreira, 1993, 1995; Adams, 2003), together with rapid growth and short life spans (Ferreira & Russ, 1992, 1994; Heupel *et al.*, 2010). Sexual pattern in *P. areolatus* has been described as protogynous (Williams *et al.*, 2008b), although histological evidence of sex change was lacking, leaving its sexual pattern unresolved.

Throughout its range, *P. areolatus* is targeted among local subsistence and large and small-scale commercial fishers (Rhodes & Sadovy de Mitcheson, 2012), including during reproductive periods (Rhodes & Tupper, 2008; Williams *et al.*, 2008a; Hamilton *et al.*, 2012). In Pohnpei, Micronesia, the species appeared in 24% of speared catches in 2006 (Rhodes *et al.*, 2008) and comprised *c.* 12% of the total grouper landings (Rhodes & Tupper, 2007). During reproductive months open to fishing, gravid adults saturate local Pohnpei markets as fishers target spawning sites (Rhodes & Tupper, 2008). At other times, juveniles dominate the catch. The need for comprehensive management across all life-history stages is evident (Rhodes *et al.*, 2008).

The objectives of this study were to establish aspects of the life history of *P. areolatus* useful for management decision-making. These include sexual pattern, size and age at sexual maturity, growth, longevity and mortality. This study coupled histological and sex-specific age-based assessments to help confirm the sexual pattern of the species, as (1) previous efforts to determine the sexual pattern of the species using histological evidence alone were inconclusive and (2) because of the inherent rarity of transitional individuals in some sex-changing groupers.

## MATERIALS AND METHODS

Samples of *P. areolatus* derived from reefs throughout the island were collected from fish markets (Fig. 1) in Pohnpei, Micronesia (6° 55' N; 158° 15' E), between 21 January 2006 and 31 January 2007. Additional specimens were obtained from recaptured individuals following a 2005 tag-recapture study of adults at a semi-protected spawning aggregation site (Kehpara Marine Sanctuary, Kehpara) during aggregation periods (Rhodes & Tupper, 2008). In Pohnpei, market and aggregation samples included fish from 244 mm total length ( $L_T$ ), while field trials captured small fish of <205 mm  $L_T$ . To fill this size distribution gap (205–244 mm  $L_T$ ) and further constrain the growth curve, this study included aged samples of

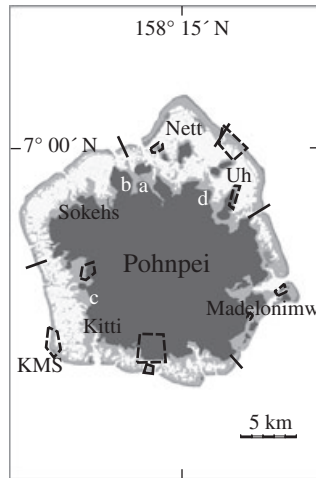


FIG. 1. Pohnpei, Micronesia showing municipal boundaries (l) and the Kehpara Marine Sanctuary (KMS). Additional sites for market sampling are listed as: (a) Kolonia, (b) Sokehs Municipality, (c) Seinwar, Kitti and (d) Uh Municipality. □, marine sanctuary sites.

juvenile *P. areolatus* (79–266 mm  $L_T$ ,  $n = 88$ ) collected during a separate study in Manus, Papua New Guinea (Almany *et al.*, 2010). Individuals from both locations were weighed whole ( $\pm 100$  g) and measured to standard ( $L_S$ ) and total ( $L_T$ ) lengths ( $\pm 1$  mm) prior to gonad (Pohnpei only) and otolith extraction.

## REPRODUCTIVE DEVELOPMENT AND SIZE AT SEXUAL MATURITY

For Pohnpei samples, gonads were extracted, weighed ( $\pm 0.1$  g) and preserved in 10% formalin for a minimum of 1 month before transfer to 70% ethanol. No gonads were taken from Manus samples. Following fixation, a sub-sample of the gonad was taken for staining (eosin, haematoxylin), processing (Leica TP1050 tissue processor; [www.leica.com](http://www.leica.com)) and transverse sectioning (7  $\mu$ m) (Louisiana State University, Department of Pathobiological Sciences, Baton Rouge).

Gonad development stages were assigned to each sample based on modified criteria for the camouflage grouper *Epinephelus polyphekadion* (Bleeker 1849) (Rhodes & Sadovy, 2002). For females, microscopic oocyte development stages were 1, chromatin nucleolar stage; 2, perinucleolar stage of development to yolk vesicle or cortical alveolar formation; 3, yolk granule (early) through migratory nucleus (late) stage, vitellogenic development; 4, hydration and 5, post-ovulatory follicle stage. Gonads of immature individuals (F1) had a thin tunica and nucleolar and perinucleolar oocytes only, while developing females (F2, developing) also contained cortical alveolar oocytes. Stages 1–3 oocytes were present in maturing (F3) female gonads, while in ripe (F4) females hydrated oocytes were also present. Spent (F5) females and mature, resting females (F2, resting) were characterized by a thickened tunica and the presence of muscle bundles, indicating prior spawning (Shapiro *et al.*, 1993; Rhodes & Sadovy, 2002). Post-ovulatory follicles in spent females indicated recent spawning (within c. 24–48 h). Only F4 and F5 individuals were considered reproductively active, while F2 resting and stages F3–F5 females were considered mature.

For males, developmental stages were determined by estimating the relative percentage of stroma (connective tissue), spermatocytes or sperm within photomicrographic sections of the gonad (Rhodes & Sadovy, 2002). Inactive male gonads (M1) were largely stroma-filled with <1% spermatocytes or sperm. Gonads of developing and mature, resting (M2) males

contained 1–10% spermatocytes or sperm, whereas 10–50% spermatocytes or sperm were found in mature (M3) male gonads. Mature, ripe (M4) males were identified as having >50% spermatocytes and sperm, while spent (M5) males had emptied seminiferous vesicles and sperm sinuses containing relatively few or atretic spermatids. In spent males, muscle bundles were often present to indicate prior spawning.

Estimates of size and age at 50% female maturity were derived by plotting the proportion of mature females by length classes, and a logistic curve was fitted to the data as follows:

$$P_R = \{1 + e^{[-\ln(19)(L_{95}-L_{50})]}\}^{-1},$$

where  $P_R$  is the estimated proportion of mature females at a given length ( $L$ ) and  $L_{50}$  and  $L_{95}$  are the lengths at 50 and 95% maturity. Curves were fitted using non-linear least-squares estimation and the corresponding 95% C.L. for each were derived by bootstrap resampling (1000 iterations). The same equation was used to determine age at female maturation and the proportion of males by length and age.

## SEXUAL PATTERN

Determinations of sexual pattern followed criteria outlined by Sadovy de Mitcheson & Liu (2008). For this study, findings were based on microscopic examination of sectioned gonads (Pohnpei) and analysis of otolith samples taken from both Manus and Pohnpei.

## REPRODUCTIVE SEASONALITY AND AGGREGATION DENSITY

The seasonal periodicity of fish spawning aggregation formation was determined by underwater visual census (UVC) conducted monthly (March 2001 to June 2002) and seasonally (January to April 2003 and 2004) by trained divers at the Kehpara *P. areolatus* fish spawning aggregation site (Fig. 1). Between 2005 and 2010, UVC was conducted during March and April only. For density estimates, total fish counts (mean  $\pm$  S.E. transect densities) were made over 2 days on new and full-moon periods using two transects (100 m  $\times$  10 m) at 15 and 30 m, respectively. Dives around new moon occurred in 2001 and 2002 only, with dives around full moon made in all years. Transect placement and depths were determined following an area assessment of the entire fish spawning aggregation in 2001, which found fish aggregating from 10 to 60 m depth and along c. 520 m of reef. For fish spawning aggregation monitoring following the initial 2001 assessment, transects were positioned from the northern edge of the fish spawning aggregation (marked with a surface buoy) through the aggregation core, which altogether covered c. one-fifth of the total aggregation. UVC monitoring started in March 2001 and was divided into deep and shallow dives made over 2 days (3 and 2 days prior to new and full moon, respectively). Three days prior to new and full moon, fish counts were made along the outer reef wall during dive 1 from 30 to 40 m depth, with dive 2 made along the 15 m transect placed at the seaward edge of the reef flat. Counts during dive 2 were made 10 m inward from the transect line towards the reef crest. Dive 3 was made 2 days prior to full moon from 30 to 20 m depth along the wall. In total, transects covered 3000 m<sup>2</sup> of the fish spawning aggregation.

Sex-specific arrival and departure times of acoustically tagged fish, and preliminary information on seasonal and lunar aggregation periodicity were reported by Rhodes & Tupper (2008). Detailed examinations of aggregation abundance and density within the fish spawning aggregation were conducted in 2011 using technical diving techniques as part of a separate study and covered over 650 m of the reef from c. 15 to 50 m depth (E. Kadison, R. S. Nemeth & K. L. Rhodes, unpubl. data). Technical dives confirmed that transect placement for this study represented both edge and core areas (*i.e.* areas of varying fish density within the fish spawning aggregation).

Reproductive seasonality was confirmed by combined UVC, microscopic gonad assessments and the gonado-somatic index ( $I_G$ ):  $I_G = 100 M_G M_T^{-1}$ , where  $M_G$  is the gonad mass and  $M_T$  is the total ungutted mass.

## AGE AND GROWTH

A sub-sample of market and recaptured fish and all Pohnpei samples of <205 mm  $L_T$  were used for age and growth assessments. Sub-samples included fish taken throughout the year and individuals from each size class. One sagittal otolith from each otolith pair was weighed and sectioned using a Buehler low-speed isomet saw ([www.buehler.com/equipment/sectioning-equipment/precision-diamond-wafering-saws/isomet-low-speed-saw](http://www.buehler.com/equipment/sectioning-equipment/precision-diamond-wafering-saws/isomet-low-speed-saw)). Thin sections (150  $\mu$ m) were taken through the core, mounted on a clear glass slide and ground using wet and dry paper as necessary. Otoliths were examined on three separate occasions by a single experienced reader and assigned an age when two or more readings matched (Russ *et al.*, 1998). Length-at-age data were fitted to the von Bertalanffy growth function (VBGF) using non-linear least-squares procedures to estimate sex-specific growth parameters. The VBGF is represented by:  $L_t = L_\infty \{1 - \exp[-K(t - t_0)]\}$ , where  $L_t$  is the  $L_T$  of a fish at age  $t$ ,  $L_\infty$  is the mean asymptotic  $L_T$ ,  $K$  is the growth coefficient which describes the rate at which fish grow towards  $L_\infty$ ,  $t$  is the age of the fish and  $t_0$  is the theoretical age at which  $L_T$  is equal to zero, as described by the growth rate. In addition to Pohnpei samples of <205 mm  $L_T$ , data for 88 unsexed individuals of age <1 year from Manus Island, Papua New Guinea, were included in order to fill size (and possible age) gaps within the growth curve. Differences in size at age were compared between sexes using ANCOVA, with age as the covariate and sex as a fixed factor. Annual instantaneous mortality rate ( $Z$ ) was estimated from age distributions using age-based catch curves. Here, the natural logarithm of the number of fish in each age class was plotted against its corresponding age and  $Z$  was estimated as the absolute value of the slope from a fitted line (Ricker, 1975).

## RESULTS

### REPRODUCTIVE DEVELOPMENT AND SIZE AT SEXUAL MATURITY

Of the 464 *P. areolatus* collected in Pohnpei, 330 were examined microscopically to determine reproductive life history. Females were 244–670 mm  $L_T$  overall (mean  $\pm$  S.E. =  $406.4 \pm 4.6$  mm  $L_T$ ,  $n = 241$ ), whereas mature females were  $429.9 \pm 4.1$  mm  $L_T$  (Fig. 2). Mature males ranged from 350 to 599 mm  $L_T$  (mean  $\pm$  S.E. =  $513.8 \pm 4.6$  mm  $L_T$ ,  $n = 88$ ) and immature males averaged  $310.3 \pm 5.4$  mm  $L_T$  (Pohnpei only). Males were on average significantly larger than females (Kruskal–Wallis, d.f. = 1,  $P < 0.001$ ); however, females were represented within the upper size classes.

Among females, immature F1 [Fig. 3(a)] and F2 mature, resting individuals [Fig. 3(c)] were present throughout most of the year [Fig. 4(a)]. F2 developing females [Fig. 3(b)] were found only 1 month prior to and during the reproductive season. The presence of stage F3–F5 females [Fig. 3(d)–(f), respectively] characterized the reproductive season (February to June 2006, January 2007) [Fig. 4(a)]. Although no reproductively active females were sampled in 2007, nearly all females were F3 in January, suggesting an interannual shift in the onset of reproductive activity. Synchronous development of hydrated oocytes was observed in all F4 ripe females [Fig. 3(e)]. Although the frequency of egg release during a spawning period is unknown, F5 females [Fig. 3(f)] were largely devoid of any residual hydrated or late-stage vitellogenic oocytes, *i.e.* all or nearly all hydrated oocytes appear to be released during a spawning month. Some presumably mature, resting females in the uppermost size classes were classified as F1 immature. For these females, muscle bundle atresia and a thinning of the gonad wall had obviously occurred prior to

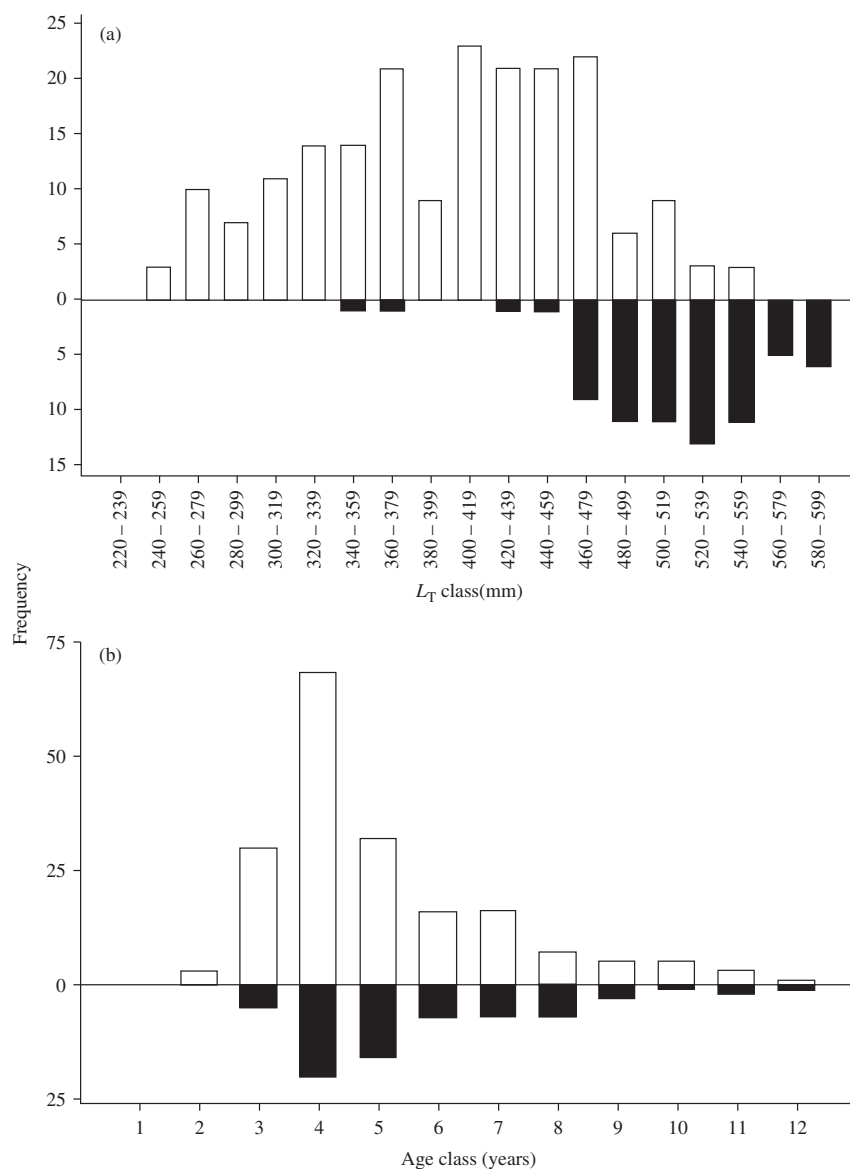


FIG. 2. (a) Total length ( $L_T$ ) and (b) age-frequency distribution of *Plectropomus areolatus* [females (□) and males (■)] sampled from local fish markets, including several recaptured tagged individuals from a 2005 tag–recapture study.

sampling. The minimum size of a mature female was 300 mm  $L_T$ , while 50% sexual maturity (2.3 years) was 366 mm  $L_T$  (Fig. 5).

Inactive M1 males [Fig. 6(a)] were observed during non-reproductive months and two aggregation months (April to May 2006) [Fig. 4(b)]. Developing and mature, resting (M2) males [Fig. 6(b)] were rare (10%) and occurred primarily outside the spawning season. Mature (M3) males [Fig. 6(c)] were first observed 1 month prior to the initial aggregation period, while mature, ripe (M4) males [Fig. 6(d)] and spent



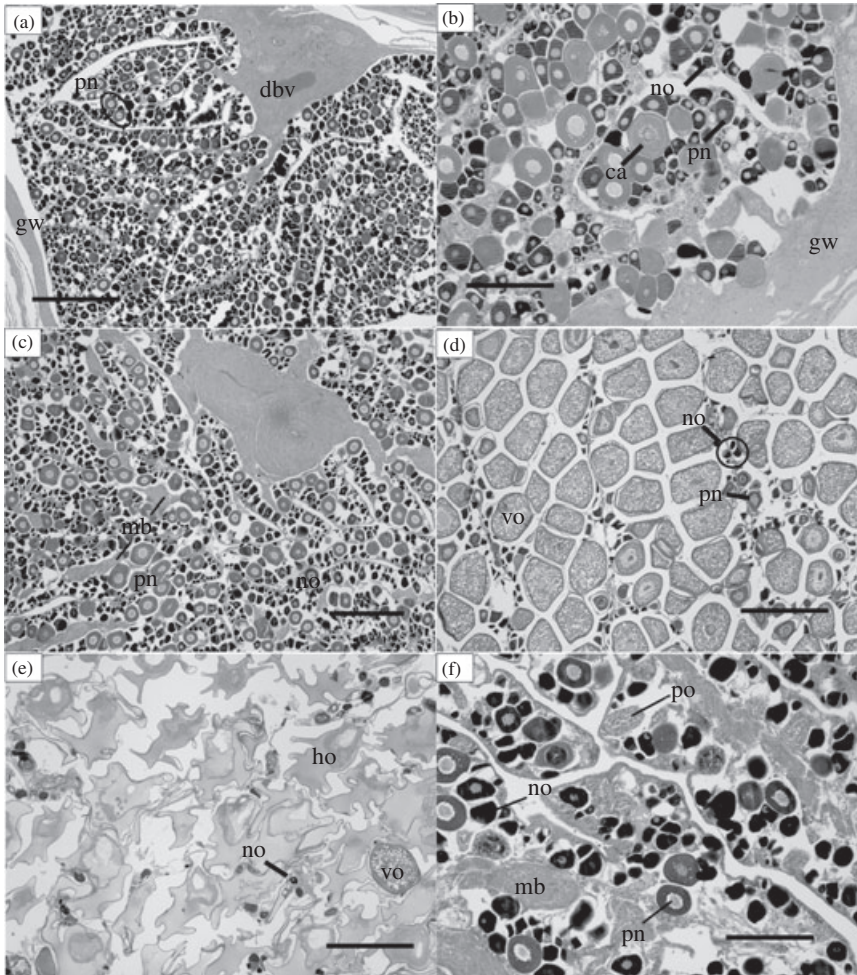


FIG. 3. *Plectropomus areolatus* female sexual development stages: (a) immature, F1 stage female (372 mm total length,  $L_T$ , May 2006; scale = 1250  $\mu$ m), (b) developing, F2 stage female (368 mm  $L_T$ , January 2006; scale = 500  $\mu$ m), (c) mature, resting F2 stage female (428 mm  $L_T$ , April 2006; scale = 1250  $\mu$ m), (d) mature, F3 stage female (440 mm  $L_T$ , January 2007; scale = 1250  $\mu$ m), (e) ripe F4 stage female (375 mm  $L_T$ , February 2006; scale = 1250  $\mu$ m) and (f) spent, F5 stage female (520 mm  $L_T$ , April 2006; scale = 500  $\mu$ m). ca, cortical alveolus; dbv, dorsal blood vessel; gw, gonad wall; ho, hydrated oocyte; mb, muscle bundle; no, nucleolar oocyte; pn, perinucleolar oocyte; po, postovulatory follicles; vo, vitellogenic oocyte.

(M5) males [Fig. 6(e)] were only found from February to May [Fig. 4(b)]. More than half (54.8%) of all the inactive males (M1) contained chromatin nucleolar or perinucleolar oocytes, including some in the upper size classes. No males of age <3 years and 350 mm  $L_T$  were observed.

## SEXUAL PATTERN

Evidence of sexual transition (as the presence of mature male tissue in the presence of late-stage, atretic oocytes) (Sadovy de Mitcheson & Liu, 2008) was absent.

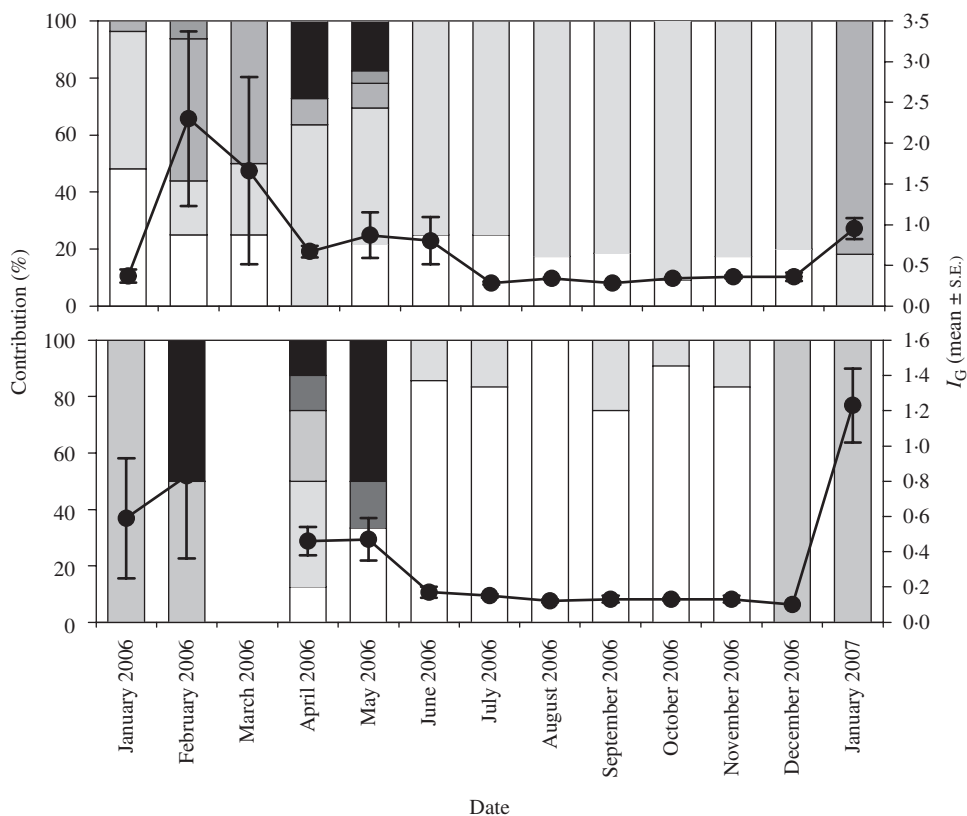


FIG. 4. Relative per cent contribution and mean  $\pm$  S.E. gonado-somatic index ( $I_G$ , -.-) of *Plectropomus areolatus* (a) females ( $n = 242$ ) by gonad development stage [F1 = immature ( $\square$ ); F2 = developing or resting, mature ( $\square$ ); F3 = mature ( $\square$ ); F4 = ripe ( $\blacksquare$ ); F5 = spent ( $\blacksquare$ )] and month (January 2006 to January 2007) and (b) males ( $n = 80$ ) by gonad development stage [M1 = inactive ( $\square$ ); M2 = developing and resting, mature ( $\square$ ); M2 = mature ( $\square$ ); M4 = mature, ripe ( $\blacksquare$ ); M5 = spent ( $\blacksquare$ )] and month (January 2006 to January 2007).

Early stage spermatocytes were observed among atretic vitellogenic oocytes in one individual (463 mm  $L_T$ ) but considered insufficient to warrant a designation of protogyny. Mature males were absent in the lowest size and age classes (age <3 years), and only two males were observed below 428 mm  $L_T$  (350 and 373 mm  $L_T$ ) (Figs 2 and 7). Although sexual transition was not confirmed, sex change ogives demonstrated an  $L_T$  at 50% sex change at 494 mm and an asymptotic sex ratio of 37% males across age classes (Fig. 7). Only three females of age 2 years were present and no gonads were available from younger fish.

## REPRODUCTIVE SEASONALITY

The UVC assessments identified aggregation formation predominantly between January and May. Interannual variability in peak density was observed (Fig. 8), with peaks observed six times in March and four times in April between 2001 and 2010. Mean density values between these months were not significantly different



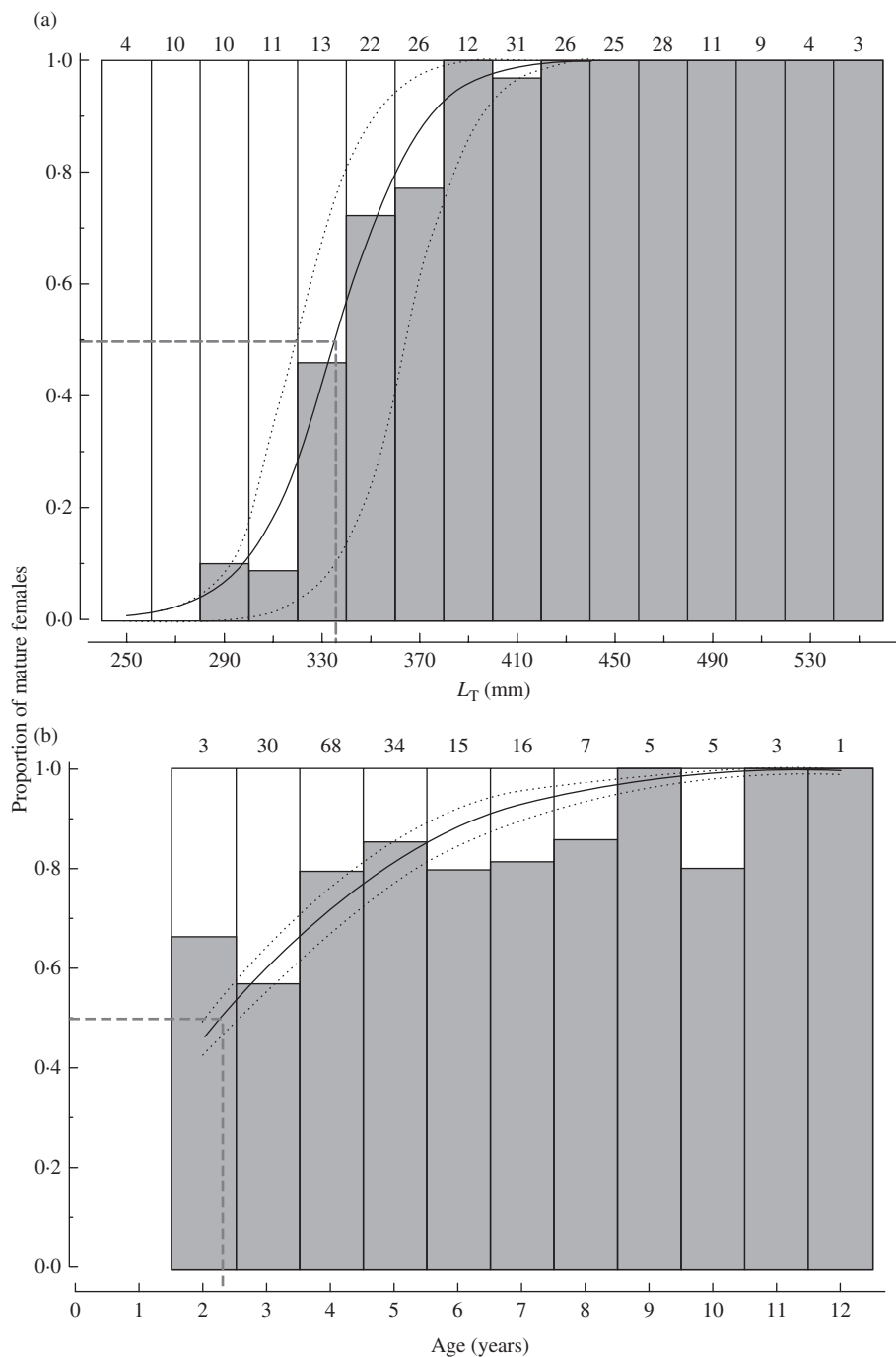


FIG. 5. Proportional frequencies of immature (□) and mature (■) female *Plectropomus areolatus* from Pohnpei by (a) total length ( $L_T$ ) and (b) age. —, the best-fit logistic equation describing the rate of maturation; ·····, 95% C.L. derived through bootstrap re-sampling; --- and |, the point of 50% (a)  $L_T$  and (b) age at sexual maturity. Sample sizes are shown for each  $L_T$  and age.

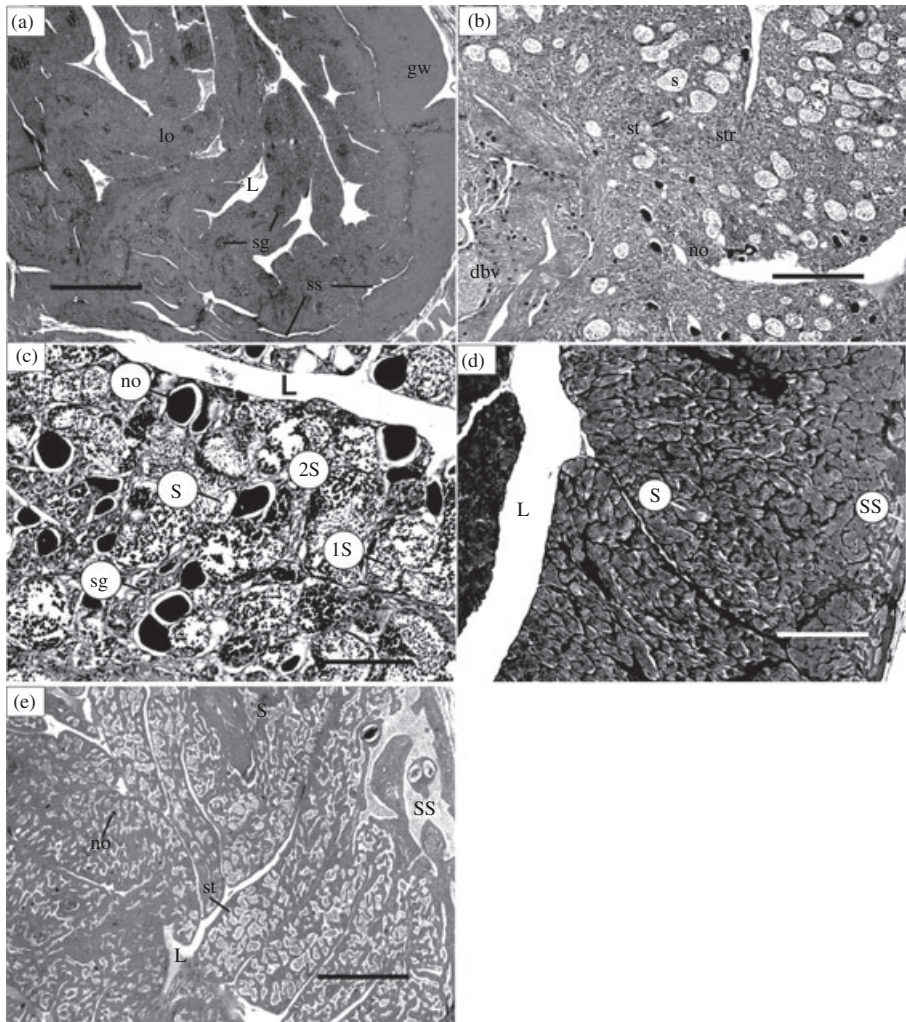


FIG. 6. *Plectropomus areolatus* male sexual development stages: (a) Immature or inactive, M1 stage male (537 mm total length,  $L_T$ , June 2006; scale = 500  $\mu$ m), (b) developing or resting, M2 stage male (490 mm  $L_T$ , October 2006; scale = 500  $\mu$ m), (c) mature, M3 stage male (497 mm  $L_T$ , January 2007; scale = 250  $\mu$ m); (d) Ripe, M4 stage male (459 mm  $L_T$ , April 2006; scale = 1250  $\mu$ m) and (e) spent, M5 stage male (466 mm  $L_T$ , April 2006; scale = 1250  $\mu$ m). 1S, primary spermatocytes; 2S, secondary spermatocytes; dbv, dorsal blood vessel; gw, gonad wall; L, lumen; lo, lobule; no, nucleolar oocyte; S, spermatids; sg, spermatogonia; SS, sperm sinus; st, seminiferous tubules; str, stroma.

( $t$ -test,  $P > 0.05$ ).  $I_G$  values over the sample period suggested that the onset of the reproductive season may occur in either January or February, *i.e.* interannual variability (Fig. 4).

## AGE AND GROWTH

Age and growth was assessed from 263 individuals from Pohnpei that included market, tag-recapture and field-derived samples. Both males and females reached

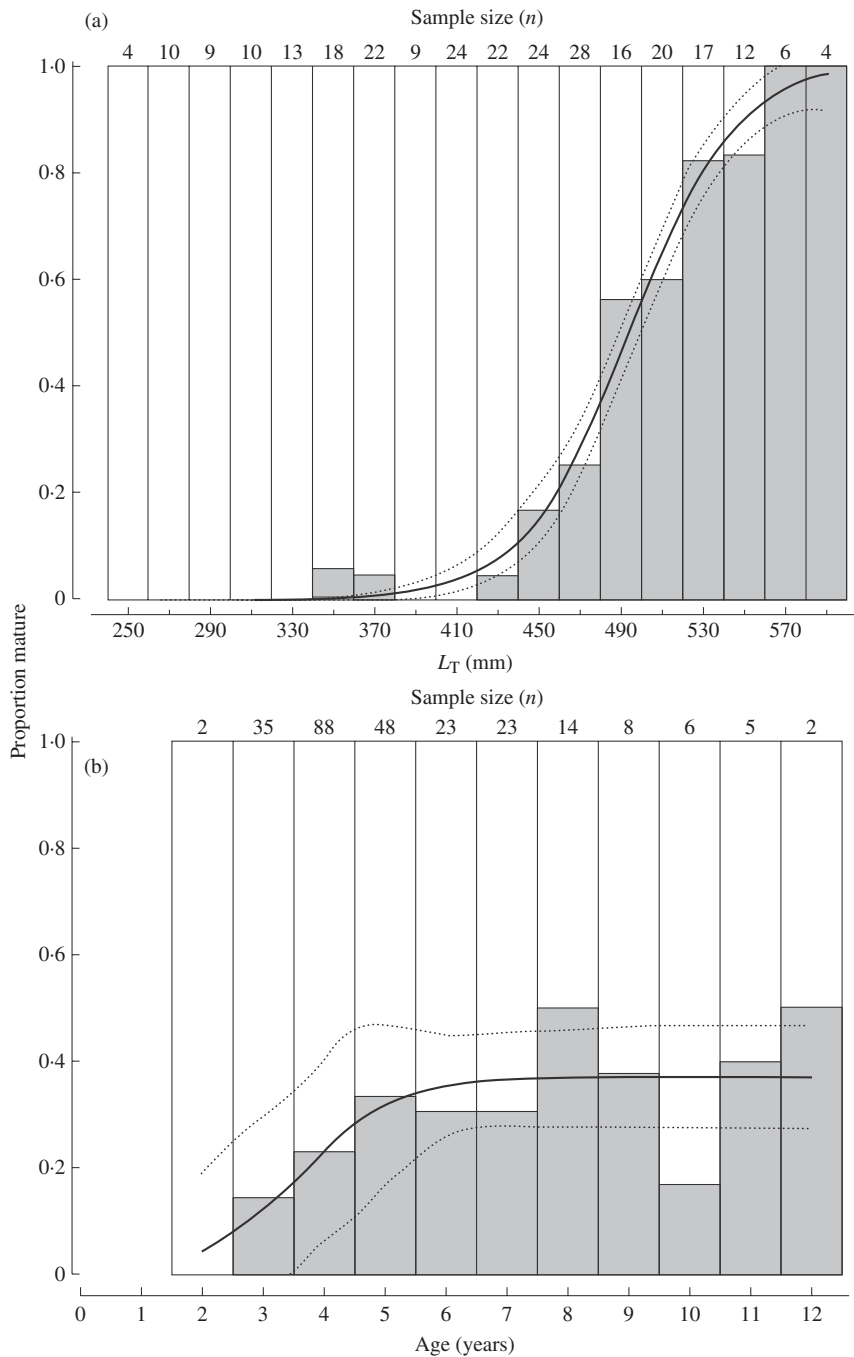


FIG. 7. Proportional frequencies of immature (□) and mature (■) *Plectropomus areolatus* from Pohnpei by (a) total length ( $L_T$ ) and (b) age. —, the best-fit logistic equation describing the rate of maturation; ..... , 95% C.L. derived through bootstrap re-sampling.

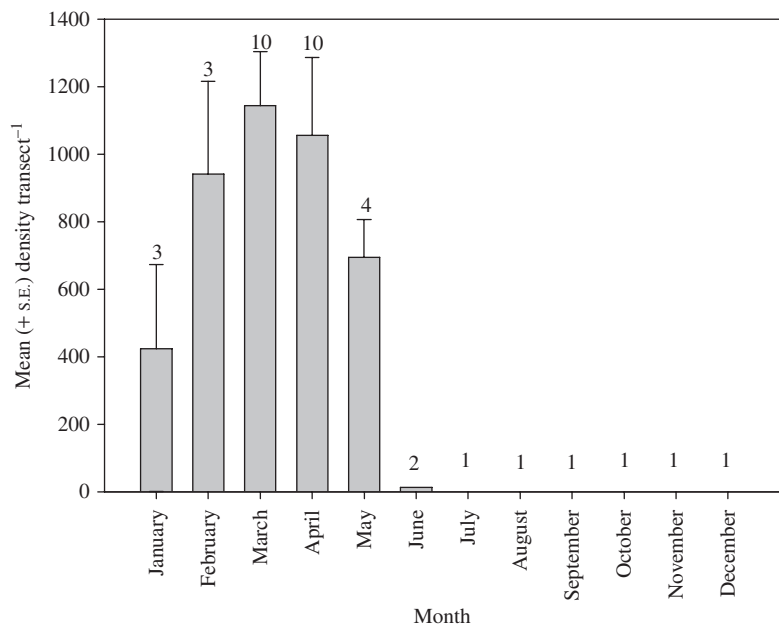


FIG. 8. Monthly mean + S.E. fish density per transect (1000 m<sup>2</sup>, averaged across three transect dives per month) of *Plectropomus areolatus* taken from underwater visual census (UVC) counts conducted from 2001 to 2010 at the Kehpara Marine Sanctuary. Monthly sample numbers for UVC monitoring periods are shown above each month.

a maximum age of 12 years (Fig. 2). Females were 2–12 years, while males were 3–12 years. Pohnpei samples of <205 mm  $L_T$  were 113–198 days old, while those from Manus ranged from 89 days (79 mm  $L_T$ ) to 359 days (266 mm  $L_T$ ). Despite a similarity in the age frequency distributions between sexes, males were significantly older than females (5.7 v. 5.0 years;  $F_{1,253} = 5.86$ ,  $P < 0.05$ ) (Fig. 2). Within all the sex-designated age classes for Pohnpei,  $L_T$  at age was highly variable as a result of sex-specific size-at-age differences, whereby males were significantly larger than females ( $F_{1,252} = 148.1$ ,  $P < 0.001$ ). Estimates of growth parameters from the VBGF for sexes pooled were  $K = 0.635 \text{ year}^{-1}$ ,  $L_\infty = 454.8 \text{ mm } L_T$  and  $t_0 = -0.309 \text{ years}$  (Fig. 9). The age-based catch curve yielded estimated  $Z = 0.428 \text{ year}^{-1}$  (Fig. 10) and suggested that *P. areolatus* was fully recruited to the fishery at age 4 years.

## DISCUSSION

By combining information from gonad and otolith analyses, *P. areolatus* was identified as a fast-growing, short-lived and early maturing epinephelid. Although mature females were observed at an earlier age than males, they were few in number. This combined with an absence of transitional individuals presented obstacles for confirming sexual pattern. In Pohnpei, females achieve 50% size of sexual maturity ( $L_{50}$ ) by age 2 years and at 366 mm  $L_T$ , while males were first identified in samples at age 3 years and 350 mm  $L_T$ . Males grew faster than females and attained significantly

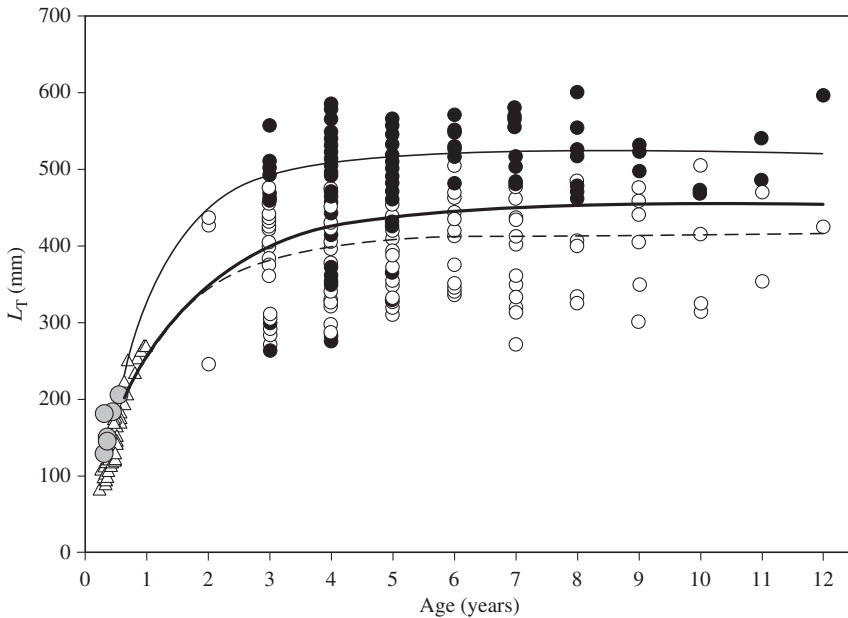


FIG. 9. Total length ( $L_T$ )-at-age plot for *Plectropomus areolatus* samples from Pohnpei. Males (●) and females (○), and juveniles of age <1 year taken from Pohnpei (●) and Manus, Papua New Guinea (△). —, best-fit von Bertalanffy growth curve for all samples combined; — —, growth curve for males; - · -, growth curve for females.

larger sizes at a given age, similar to hermaphroditic coralgrouper *Plectropomus maculatus* (Bloch 1790) (Adams & Williams, 2001).

In Pohnpei, *P. areolatus* forms seasonal aggregations over a 5 month period that differs in timing and duration from other known locales, including Palau, Papua New Guinea, Solomon Islands and eastern Torres Straits, Australia. Interannual variations in peak aggregation abundance were recorded from UVC and were consistent with gonado-somatic analyses.

## SEXUAL PATTERN

Epinephelids display a variety of sexual patterns (Sadovy de Mitcheson & Liu, 2008), with all plectropomids examined to date demonstrating protogyny: *P. maculatus* (Ferreira, 1993; 1995; Adams, 2003; Williams *et al.*, 2008b), leopard coralgrouper *Plectropomus leopardus* (Lacépède 1802) (Ferreira, 1995; Williams *et al.*, 2008b) and blacksaddled coralgrouper *Plectropomus laevis* (Lacépède 1801) (Heupel *et al.*, 2010). The only previous study to examine sexual pattern for *P. areolatus* utilized samples from the eastern Torres Strait (Australia) that were similar in both size [c. 300–640 mm fork length ( $L_F$ )] and age (2–14 years) (Williams *et al.*, 2008b) to those from this study. Based on those evaluations, *P. areolatus* was designated as protogynous, with transitionals in 18 of the 448 samples. To confirm the findings of Williams *et al.*, 2008b, three of the 18 fish reported by those authors as transitionals (size range = 525–630 mm  $L_F$ , age 5–7 years) were examined. During those examinations, no evidence of sexual transition or prior female reproductive activity



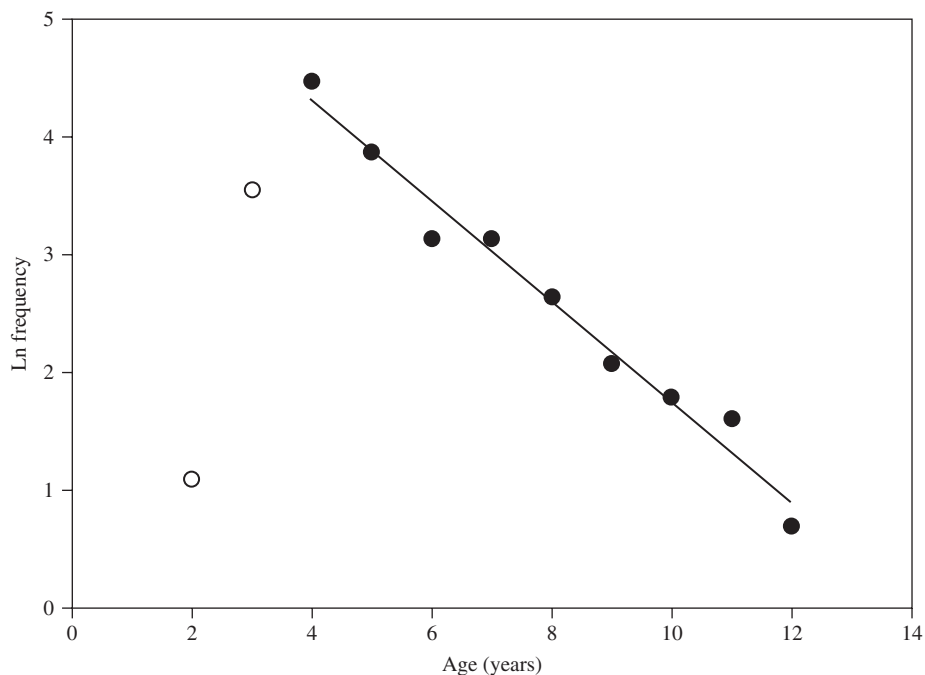


FIG. 10. Age-based catch curve for *Plectropomus areolatus* samples. Annual instantaneous total mortality rate ( $Z$ ) is represented by the absolute value of the slope of the best-fit line fitted to descending values ( $y = -0.428x + 6.026$ ;  $r^2 = 0.975$ ). Only filled points were used in the analysis.

was apparent among the specimens that were made available. Potential errors in earlier designations for these three fish open the possibility that sexual pattern is still unresolved for the species.

*In lieu* of transitionals in Pohnpei samples, this study attempted to use age-based techniques to identify sexual pattern. The techniques reported here were similar to those previously used to characterize hermaphroditic (Sadovy *et al.*, 1992; Wyanski *et al.*, 2000; Choat *et al.*, 2006; Coulson *et al.*, 2009) and gonochoristic sexual patterns (Rhodes *et al.*, 2011) in other species. Using those techniques, significant sex-specific size and age differences were identified, similar to those characterizing some protogynous species. Unfortunately, the paucity of age 2 year females and absence of gonads from young (age <2 years) individuals precluded a strong conclusion of sexual pattern. Given the lack of conclusive histological evidence from these two studies, the sexual pattern for *P. areolatus* remains unresolved.

#### DEMOGRAPHIC COMPARISON OF AGE, GROWTH AND MORTALITY

In Pohnpei, *P. areolatus* displays fast growth, early maturity and a life span and mortality schedule similar to other plectropomids (Ferreira & Russ, 1992, 1994; Adams *et al.*, 2000; Williams *et al.*, 2008b; Heupel *et al.*, 2010). Growth patterns were sex specific, with male mean maximum size exceeding that of females, and highly asymptotic, with 90% of the  $L_{\infty}$  achieved by age 3 years. The age-based catch

curve provided a strong fit to the age-frequency data, suggesting that an estimate of  $Z = 0.43 \text{ year}^{-1}$  is generally robust and compares well with previous estimates of  $0.40 \text{ year}^{-1}$  from the eastern Torres Strait (Williams *et al.*, 2008b). In contrast, asymptotic growth for *P. areolatus* in the eastern Torres Strait was reported to continue throughout the life span of the species (Williams *et al.*, 2008b). Such marked differences in growth pattern of reef fishes have been demonstrated previously on both large (Robertson *et al.*, 2005) and small spatial scales (Gust *et al.*, 2002; Kritzer, 2002; Williams *et al.*, 2003) and may reflect a response to a variety of environmental or biological factors.

The resultant difference in sex-specific sizes coupled with highly asymptotic growth creates a management concern for *P. areolatus* in Pohnpei. Size-selective targeting of large-bodied groupers is an axiom in fisheries science (Bannerot *et al.*, 1987; Jennings & Lock, 1996; Sadovy, 1996) and has been linked to decreased reproductive success where size-selective exploitation significantly alters sex ratios (Coleman *et al.*, 1996). Hence, effort controls would probably be more effective than minimum size limits as the latter may only increase exploitation pressure on large males.

## MANAGEMENT

To improve management in Pohnpei and in the absence of volume control mechanisms, the  $L_{50}$  reported here was used to develop new minimum size-at-sale regulations, which were implemented in October 2012. Likewise, UVC data provided the basis for recommending an expansion of the current March to April grouper sales ban to match the observed seasonal aggregation pattern (January through May). More recently, an ecological footprint analysis for the Pohnpei coral-reef fishery concluded that the coral-reef fishery is at least four to seven times above sustainable harvest levels, indicating that even greater pressure will be placed on *P. areolatus* as other stocks decline (K. L. Rhodes, K. A. Warren-Rhodes, S. Sweet, M. Helgenberger, E. Joseph & K. D. Hopkins, unpubl. data). Based on these combined results, the new management measures recently implemented are timely. Additional management is needed to reduce harvest quantities to sustainable levels, with stronger enforcement of existing regulations.

This project was supported by NOAA Coral Reef Conservation Grants to the University of Guam (NA05NMF4631049 and NA04NMF4630341) and grants to the Conservation Society of Pohnpei by the U.S. Department of the Interior Office of Insular Affairs, the David and Lucile Packard Foundation and U.S. Fish and Wildlife Service Pacific Islands Coastal Programme. Monitoring training was financed through a grant to The Nature Conservancy from the David and Lucile Packard Foundation and the Sea Grant College Programme. Initial monitoring assistance was provided by U.S. Peace Corps volunteers. James Cook University School of Marine and Tropical Biology (D. C. Lou) was responsible for otolith processing and ageing. This research was done in accordance with the laws governing animal welfare in the Federated States of Micronesia.

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